Gut Microbiota: An Overview

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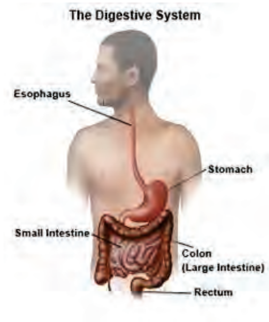
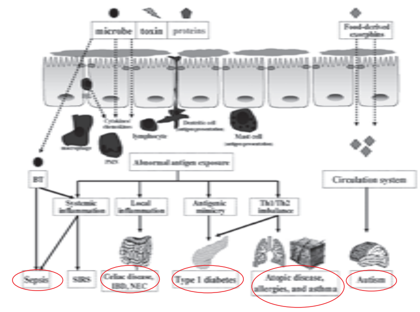
Digestive Health - Gut microbiota



- Our gastrointestinal system work hard to keep us healthy and happy
- When gut health is compromised, we can face major health consequences

– "Heal the gut and you heal yourself." –

What might happen in the body when gut health is compromised ?



Anxiety
Depression

Obesity

Skin disaeses

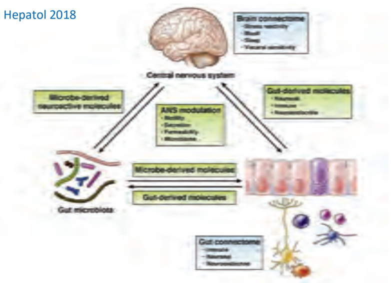
The Brain-Gut-Microbiome Axis

Clair R. Martin, Vadim Osadchiy, Amir Kalani, and Emeran A. Mayer

G. Oppenheimer Center for Neurobiology of Stress and Resilience, Vatche and Tamar Manoukian Division of Digestive Diseases, Microbiome Center, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, California

Cell Mol Gastroenterol Hepatol 2018

- A healthy immune system
- Intact mucosa (barrier)
- Balanced intestinal bacteria



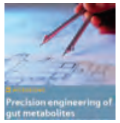
Systems biological model of brain-gut-microbiome interactions

- Brain-Gut Axis
- **Brain-Gut-Microbiota Axis**
- Gut-Lung Axis
- Gut-Joint Axis
- Gut-Skin Axis

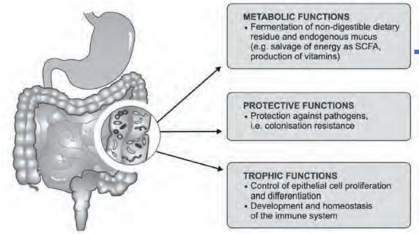
- Over the last decade, the gut microbiome is one of the most popular research topics
- Gut microbiota has huge immunological impact and metabolic capacity which may affect other organ including the skin, and hence it is central to the gut-skin axis

Gastrointestinal microbiota: *a complex ecosystem*

-We have more bacteria in our gut than cells in our body-



Intestinal flora is useful for us



Metabolites (byproducts)
- SCFAs: acetic-, propionic-, butyric acids
- Trimethylamine N-oxide (TMAO)
- Free phenol and p-cresol

Methods for microbiota analysis

The method of choice is defined by the raised question

Culture

Next gen sequencing

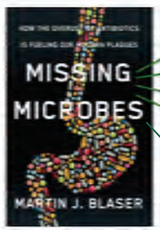
PCR

Nanopore sequencing

- offer relatively low-cost genotyping
- high mobility for testing
- rapid processing of samples with the ability to display results in real-time

Nature Biothechnology 2019

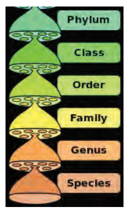
Factors affecting the microbiome balance



- Increased elective Cesarean section
- Decreased breastfeeding
- (Over)use of antibiotics
- Western diet (high in fat, animal protein, and simple sugars, low in complex carbohydrates)

Other factors:
Gut pH, infection, GI motility

Dominant microbimes



Phylum Bacteroidetes
- Gram-negative, anaerobic, rod-shaped bacteri
- Major genera: Bacteroides, Prevotella

Phylum Firmicutes
- Mostly Gram-positive, (facultative) anaerobic bacteria
- Many Firmicutes produce endospores
- Major genera: Lactobacillus, Clostridium, Ruminococcu

Phylum Proteobacteria
- Gram-negative, mostly (facultative) anaerobic bacte
- This phylum includes many medically relevant pathogens
- Major genera: Escherichia, Desulfovibrio

Phylum Actinobacteria
- Gram-positives
- Major genus: Bifidobacterium

Phylum Verrucomicrobia
- One main species present in the intestinal tract: *Akkermansia mucinophila*

Table 1. Overview of Papers Studying the Microbiota of the Gut with Regards to Irritable Bowel Syndrome

Phyla	Genus	IBS against healthy
Firmicutes	*Lactobacillus*	↑ Tana (2010); Carroll (2010); Carroll (2011)
		↓ Balsari (1982); Kassinen (2007)
		↔ Si (2004); Malinen (2005); Kerckhoffs (2009); Rajilić-Stojanović (2011)
	Streptococcus spp.	↑ Kassinen (2007); Rajilić-Stojanović (2011)
	Dorea spp.	↑ Saulnier (2011); Rajilić-Stojanović (2011); Kassinen (2007)
	Ruminococcus spp.	↑ Kassinen (2007); Rajilić-Stojanović (2011); Jalanka-Tuovinen (2014)
	Eubacterium	↔ Rajilić-Stojanović (2011)
	Faecalibacterium	↔ Lopez-Siles (2014)
Actinobacteria	*Bifidobacterium*	↑ Kassinen (2007)
		↔ Balsari (1982); Si (2004); Kerckhoffs (2009); Rajilić-Stojanović (2011); Malinen (2005); Duboc (2012)
Bacteroidetes	*Bacteroides* spp.	↑ Jalanka-Tuovinen (2014)
		↓ Compared to IBD Swidsinski (2005)
Proteobacteria	*Escherichia*	↑ Rajilić-Stojanović (2011); Duboc (2012); Si (2004)
	Desulfovibrio spp.	↓ Malinen (2005)
Verrucomicrobia	*Akkermansia*	↑ Saulnier (2011)
		↔ Rajilić-Stojanović (2011)
Euryarchaeota	*Methanobrevibacter*	↓ Rajilić-Stojanović (2011); Jalanka-Tuovinen (2014); Rana (2009)

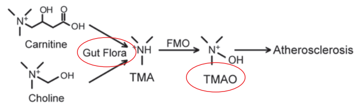
Microbial metabolites or components that are implicated in disease

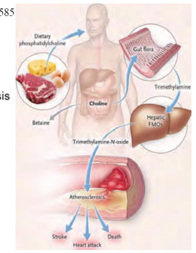
Diseases	Microbial metabolites or components
Gastrointestinal disorders	
Inflammatory bowel disease	SCFAs, B vitamins
Irritable bowel disease	SCFAs
Infectious colitis (*Clostridium difficile*)	Bile acids
Cancer	
Colorectal cancer	SCFAs, N[1],N[12]- diacetylspermine
Metabolic disorders	
Cardiovascular disease	TMAO
Kidney disease	p-Cresol, p-Indoxyl, TMAO
Obesity	TMAO
Diabetes mellitus type 2	TMAO
Neurologic disorders	
Autism spectrum disorders	4-ethyl phenol sulphate
Central nervous system dysfunction	SCFAs

Intestinal microbiota metabolism of *L*-carnitine, a nutrient in red meat, promotes atherosclerosis

Robert A. Koeth[1,2], Zeneng Wang[1,2], Bruce S. Levison[1,2], Jennifer A. Buffa[1,2], Elin Org[3], Brendan T. Sheehy[1], Earl B. Britt[1,2], Xiaoming Fu[1,2], Yuping Wu[4], Lin Li[1,2], Jonathan D. Smith[1,2,5], Joseph A. DiDonato[1,2], Jun Chen[6], Hongzhe Li[6], Gary D. Wu[7], James D. Lewis[6,8], Manya Warrier[9], J. Mark Brown[9], Ronald M. Krauss[10], W. H. Wilson Tang[1,2,5], Frederic D. Bushman[5], Aldons J. Lusis[3], and Stanley L. Hazen[1,2,5]

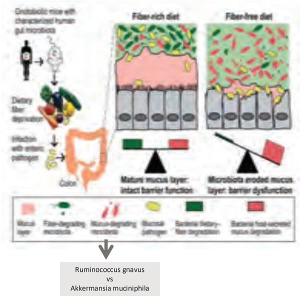
Nat Med. 2013 May ; 19(5): 576–585





- Trimethylamine N-oxide (TMAO) is a known atherogenic molecule
- Carnitine and lecitin are converted to TMA by gut microbes, oxidized to TMAO in the liver

Veggies and Intact Grains a Day Keep the Pathogens Away



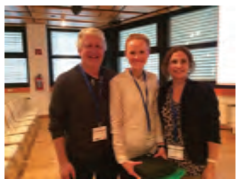
- Low-fiber diet promotes expansion of colonic mucus-degrading bacteria
- Purified prebiotics fibers do not alleviate degradation of the mucus layer
- Fiber-deprived gut microbiota promotes aggressive colitis by an enteric pathogen

Desai MS, et al. Cell 2016

Effects of varying dietary content of fermentable short-chain carbohydrates on symptoms, fecal microenvironment, and cytokine profiles in patients with irritable bowel syndrome

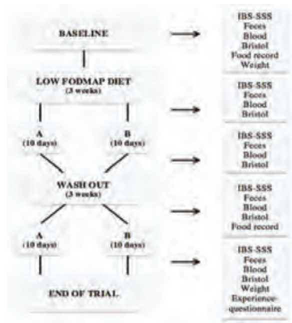
T. N. Hustoft[1] | T. Hausken[1,2,3] | S. O. Ystad[1,3] | J. Valeur[4] | K. Brokstad[5] | J. G. Hatlebakk[1,2,3] | G. A. Lied[1,2,3]

Neurogastroenterologi & Motility, 2017



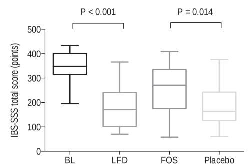
"Oral Free Paper Prize" and "National Scholar Award - Norway" in Wien (United European Gastroenterology Week), 2017

Data and material collection

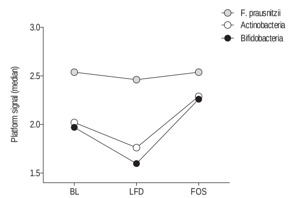


A: Fructo-oligosaccharides (FOS): (FODMAP)
B: Maltodextrin (placebo): (not a FODMAP)

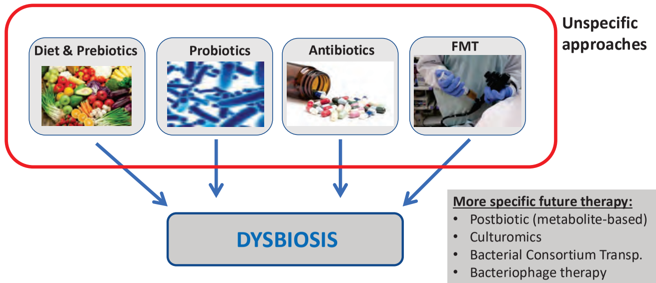
IBS-symptoms



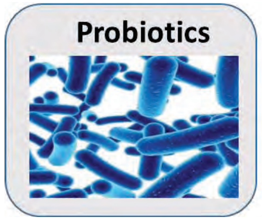
Faecalibacterium prausnitzii, Actinobacteria and Bifidobacterium abundance were significantly altered in both dietary interventions



Therapeutic Approaches for Dysbiosis



More specific future therapy:
- Postbiotic (metabolite-based)
- Culturomics
- Bacterial Consortium Transp.
- Bacteriophage therapy



" Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host "


nutrients
MDPI
Communication
Probiotics in Irritable Bowel Syndrome: An Up-to-Date Systematic Review

Hanna Fjeldheim Dale [1,2,3,*], Stella Hellgren Rasmussen [1], Özgün Ömer Asiller [1,4] and Gülen Arslan Lied [1,2,3]

1 Centre for Nutrition, Department of Clinical Medicine, University of Bergen, 5009 Bergen, Norway
2 Division of Gastroenterology, Department of Medicine, Haukeland University Hospital, 5021 Bergen, Norway
3 National Centre of Functional Gastrointestinal Disorders, Haukeland University Hospital, 5021 Bergen, Norway
4 Department of Gastroenterology, Ankara University Faculty of Medicine, Mamak Ankara 06620, Turkey
* Correspondence: hanna.dale@outlook.com

Received: 4 July 2019; Accepted: 27 August 2019; Published: 2 September 2019

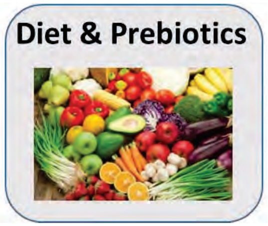
- The specific symptoms improved by probiotic supplementation were not consistent between studies
- When comparing the results of administering a multi-strain vs a mono-strain probiotic supplement, a supplement with a multi-strain probiotic has the potential to improve IBS symptoms

Predominant bacteria genera

- **In the intestinal lumen**
  - Bacteroides
  - Bifidobacterium
  - Lactobacillus
  - Streptococcus
  - Enterobacteriaceae
  - Enterococcus
  - Ruminococcus

- **In the mucosa-associated surface**
  - Akkermansia
  - Clostridium
  - Lactobacillus
  - Enterococcus

Next generation probiotics
- Akkermansia muciniphila,
- Clostridium clusters IV, XIVa, and XVIII (Clostridium leptum),
- Faecalibacterium prausnitzii,
- Bacteroides uniformis CECT 7771



Prebiotics

- The concept for using of prebiotics was first introduced in 1995

- Prebiotics are non-digestible substances taken by the human host that, when taken in adequate amounts, produce beneficial physiological effects on the host by stimulating, the growth and metabolic activity of a limited number of beneficial indigenous bacteria (bifidobacteria and lactic acid bacteria)

Artificially synthesized prebiotics

- Lactulose
- Lactosaccharose
- Fructo-oligosaccharides (FOS)
- Galacto-oligosaccharides (GOS)
- Cyclodextrins

Natural prebiotic

- Fructan
- Inulin
- Cellulose
- Hemicellulose
- Pectin

Increasing amount of evidence indicate that the ***dietary fibers acting as prebiotics, such as inulin and oligosaccharides***, are able to influence the composition of the gut microbiota, and fermentation of dietary fibers leads to the production of SCFAs

Antibiotics

Antibiotics

- Rifaximin, Neomycin or combination of both
- Rifaximin: 3 multicenter RCTs demonstrated a beneficial effect in improving IBS symptoms
  - TARGET 1
  - TARGET 2

$\}$ **IBS-D and M**, 550mg x 3 for 2 weeks, significantly reduced overall symptoms and bloating when compared to placebo (**40.8% vs. 31.2% in TARGET 1** and **40.6% vs. 32.2% in TARGET 2**)

FMT


PLOS ONE 2016

RESEARCH ARTICLE

Systematic Review: Adverse Events of Fecal Microbiota Transplantation

Sinan Wang[1*], Mengque Xu[1*], Weiqiang Wang[1], Xiaocang Cao[1], Meiyu Piao[1], Samiullah Khan[1], Fang Yan[1,2], Hailong Cao[1,2*], Bangmao Wang[1*]

Reviewing 50 publication including 16 case series, 9 case reports and 4 randomized controlled trials (RCT)

All	CDAD	IBD	Both	Other
N=1089	831	235	106	23

Adverse reactions: Common with short-term 1-2 weeks self-limiting side effects 18% (lower) vs 44% (upper GI delivery)

Serious adverse reactions: Death (aspiration), infections (virus, bacteriemi, sepsis), toxic megacolon, IBD flare, auto-immune diseases, rectal abscess after enema

Preparation and Delivery Modalities


Fresh


Frozen


Dry capsules

The routes of administration:

Colonoscopy
Retention enema
Nasogastric tube
Nasojejunal tube
Nasoduodenal tube
Gastroscopy
Sigmoidoscopy
Capsules

Consideration of stool donors (exclusion criteria) – Super donor?

Fever of unknown origin or any suspected infectious disease
Diabetes mellitus
Abnormal body mass index
Metabolic syndrome
Autoimmune diseases
Neurological diseases
Active communicable illness (HIV, chronic hepatitis, etc.)
Exposure risk for hepatitis or HIV in past 12 months
Travel to high risk areas for infectious diarrhea in past 12 months
Recent acute diarrheal illness within 6 months
Chronic diarrheal disorder including but not limited to • Irritable bowel syndrome or functional diarrhea • Inflammatory bowel disease • Microscopic colitis • Celiac disease
Chronic constipation
Known colonic malignancy or polyps
History of C. difficile infection
Hospitalization within the past 12 months
Antibiotic exposure within the past 12 months

High risk sexual behavior (men having sex with men, multiple sexual partners)
Immunosuppressive or antineoplastic medication use
Illicit drug use; recent tattoos or incarceration
Recent ingestion of known allergen to which recipient is known to be allergic
Infectious Disease Testing for Donors
Human immunodeficiency virus antibody
Syphilis enzyme immunoassay or RPR
Hepatitis A IgM
Hepatitis B surface antigen, Hepatitis B surface and core antibody levels
Hepatitis C antibody
Clostridium difficile stool assay
Bacterial enteric pathogen culture or PCR
Vancomycin resistant *Enterococcus* PCR
Ova and parasite exam
Cryptosporidia and microsporidia testing

CLINICAL PHARMACOLOGY & THERAPEUTICS | VOLUME 103 NUMBER 1 | JANUARY 2018

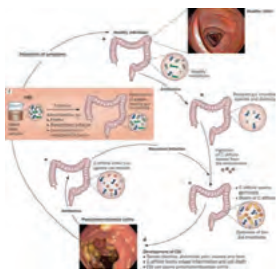
Scientific Evidence

2022 ?

- Documentation of both short- and long-term effect and safety

Only for recurrent Clost. Dif. infection

Fecal Microbiota Transplantation



Primary Indication (Clinical practice)

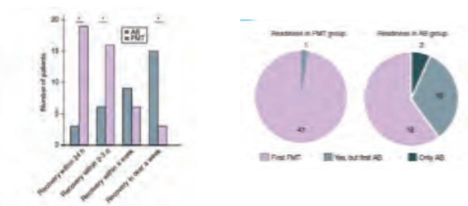
- C. Difficile infection
  - Recurrent /relapsing
  - Moderate, therapy resistant
  - Severe, therapy resistant for 48 hr

Other indications (Research)

- IBD
- IBS
- Obesity / metabolic syndrome, type 2 Diabetes, fatty liver disease
- Hepatic encephalopathy
- Pediatric allergy disorders

The long-term effects of faecal microbiota transplantation for gastrointestinal symptoms and general health in patients with recurrent *Clostridium difficile* infection

Jalanka J. Aliment Pharmacol Ther 2018



- 84 patients (45 received a FMT treatment and 39 served as controls receiving antibiotics). Duration of followed up: average for 3.8 years.
- **Conclusion:** FMT is a durable, safe and acceptable treatment option also in long term, and it shows potential benefits over antimicrobial treatment.

FMT in IBS

Varying delivery of FMT for IBS

Study	N	Bowel prep	Route	Material used / Placebo	Regimen	Trial Length weeks
Halkjaer 2018	52	✓	Oral	Frozen 25 Capsules **	Daily X 12	12
Aroniadis 2018	48	none	Oral	Frozen 25 Capsules **	Daily X 3	12
Holvoet 2021	62	✓	NG tube	300ml fresh slurry *	1X	12
El-Sahly 2020	165	none	Gastro	Frozen 30/60g 50ml*‡	1X	12
Johnsen 2018	90	✓	Colon †	Fresh/frozen 80g/200ml *	1X	12
Holster 2019	17	✓	Colon	Frozen 30g / 150ml *	1X	24
Lahtinen 2020	49	✓	Colon	Frozen 30g / 100ml * ‡	1X	52

Placebo * = autologous stool ** = inert material ‡ single donor

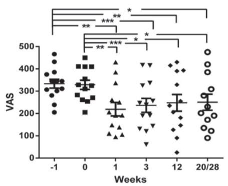
RESEARCH ARTICLE

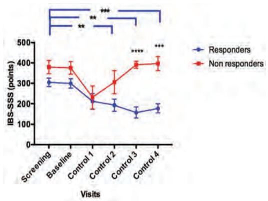
The kinetics of gut microbial community composition in patients with irritable bowel syndrome following fecal microbiota transplantation

Tarek Mazzawi[1,2,3]*, Gülen Arslan Lied[1,2,3], Dag André Sangnes[1], Magdy El-Salhy[2,3,4], Johannes R. Hov[5], Odd Helge Gilja[1,2,3], Jan Gunnar Hatlebakk[1,2,3], Trygve Hausken[1,2,3]

PLoS ONE, 14. November 2018; 13(11):e0194904

IBS-symptom severity scoring system (IBS-SSS)





Bacterial signals before and after FMT

Bacteria strain	Donors Before, n=13	Patients: Before FMT, n=9	Patients: After 1 week, n=12	Patients: After 3 weeks, n=9	Patients: After 12 weeks, n=13	Patients: After 20/28 weeks, n=12	Donors After, n=10	P* Before FMT	P** After 1 week	P*** After 3 weeks	P**** After 12 weeks	P***** After 20/28 weeks
Ruminococcus gnavus	4.6±1.1	40±15.6	8±2.3	15.3±9	25±17	8.1±1.8	8.8±2	**0.007**	>0.9	0.15	0.44	>0.9
Bacteroide	27.2±4	38.9±8	47±17	30.9±4	55±12	49±11	42±5	>0.9	>0.9	>0.9	**0.002**	0.1
Bacteroides, Prevotella	483±51.4	634±28.5	599±25.8	551±63.9	783±49.7	731±52	788±58	0.7	>0.9	>0.9	**0.005**	**0.02**
Alistipes	100±16.8	100±23	119±17	140±26.8	186±11.9	208±9.6	188±15	>0.9	>0.9	0.9	**0.011**	**0.0006**
Parabacteroides	7.6±1.8	7.9±2.9	8.3±2	14.6±5.4	11.8±1.8	15.7±3.5	19.5±3.8	>0.9	>0.9	>0.9	0.4	0.3
Proteobacteria	27±7.8	195±122	566±162	39.4±16	124±71	59.9±43	17±1.9	>0.9	**0.005**	0.69	0.11	0.77
Shigella, Escherichia	62±23	260±124	578±128	116±45	188±77	90.9±65	41±14	0.3	**0.0006**	0.2	0.2	0.9
Actinobacteria	287±45	66.6±13	95±23	197±54	138±29	92±23	204±57	**0.001**	**0.0008**	0.16	**0.012**	**0.002**
Bifidobacterium	324±57	65±13	97±25	205±57	150±34	92.5±25	241±72	**0.0011**	**0.0008**	0.12	**0.016**	**0.0012**

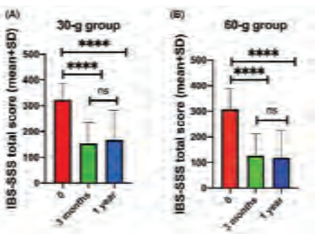
Data are presented as the mean±SEM. Comparison: Mann-Whitney U test. *Donors vs. patients before FMT, **Donors vs. patients 1 week after FMT, *** Donors vs. patients 3 weeks after FMT, ****Donors vs. patients 12 weeks after FMT, *****Donors vs. patients 20/28 weeks after FMT, ******Donors at the beginning vs. end of the study.

Mazzawi et. al. THE KINETICS OF GUT MICROBIOTA FOLLOWING FAECAL MICROBIOTA TRANSPLANTATION IN PATIENTS WITH IRRITABLE BOWEL SYNDROME. **UEG. 2016**

Long-term effects of fecal microbiota transplantation (FMT) in patients with irritable bowel syndrome

Magdy El-Salhy[1,2,3] | Anja Bråthen Kristoffersen[4] | Jørgen Valeur[5,6] | Christina Casen[4] | Jan Gunnar Hatlebakk[2,3] | Odd Helge Gilja[2,3] | Trygve Hausken[2,3]

Neurogastroenterology & Motility 2021

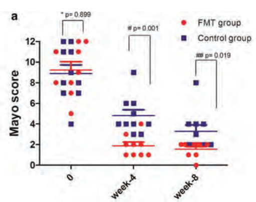


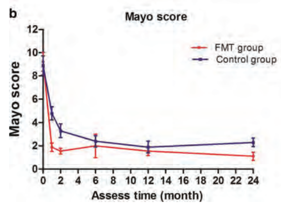
- Most patients who had responded to FMT after 3 months maintained their response after 1 year
- Their abdominal symptoms, fatigue, and QoL were improved at 1 year compared with at 3 months after FMT

Long-term efficacy and safety of monotherapy with a single fresh fecal microbiota transplant for recurrent active ulcerative colitis: a prospective randomized pilot study

Haiming Fang[1,2*], Lian Fu[1,2], Xuejun Li[3], Chunxia Lu[1,2], Yuan Su[1,2], Kangwei Xiong[1,2] and Lijiu Zhang[1,2]

Microb Cell Fact 2021





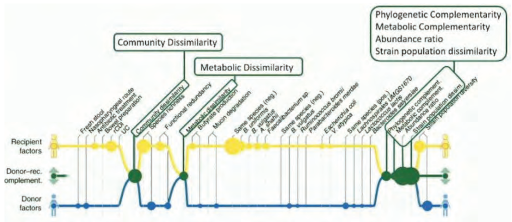
Unanswered issues for FMT

- Preperation of fecal material (fresh, frozen, capsules)
- Route of application
- Number and frequency of FMT
- Dosage of stool per FMT
- Value antibiotic pre-treatment
- Bowel lavage before FMT
- Specification of donor (related or unrelated) (single, multidonor, donor-mix, superdonor)

Claim for standardized protocols

The American Gastroenterological Association (AGA) has FMT registry ***(http://www.gastro.org/patient-care/registries-studies/fmt-registry)*** to help characterise long-term outcomes of FMT

«Complementary» between donor and recipient microbiota matters much more than donor microbiota alone



UEGW 2022

FMT - Summary

- Proven effect treatment for recurrent C difficile infection
- Results for IBS variable and may depend on donor selection
  - selection should be personalized to the recipient`s microbiota

- Need to characterise «super donors» microbiologically
- Duration of benefit in IBS uncertain
- Future studies needed to define
  - optimum donor microbiota
  - patient factors predicting effectiveness

- All patients should be in randomised placebo controlled trials (RCT)
- Investigate the role of diet in FMT
  - better response short-term or long-term?

Future therapeutic strategies

More specific future therapy:
- Postbiotic (metabolite-based)
- Culturomics
- Bacterial Consortium Transp.
- Bacteriophage therapy

Postbiotic

Pathogenic bacterial metabolites
- TMAO
- Hydrogen sulfide

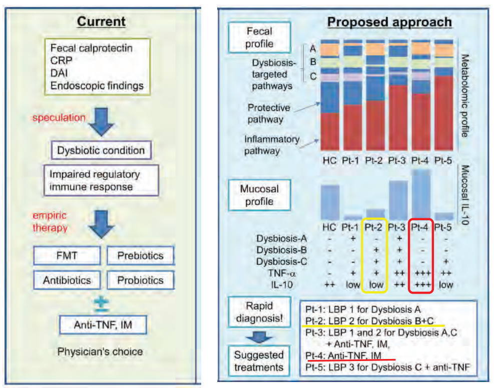
Protective bacterial metabolites
- SCFAs
- Flavonoids
- Taurine
- Indols
- Sphingolipids

Bacterial Consortum Transplantation:
(Artificial bacterial suspension)
- RePOOPulate: 33 different purified gut bacteria isolated from a healthy donor

Bacteriophage therapy
- Phages are viruses that infect bacteria, and have a great therapeutic potential
- Suspensions of phages could be used either for **antimicrobial purposes** (alternative to antibiotics) or to **modulate the composition of microbial communities**

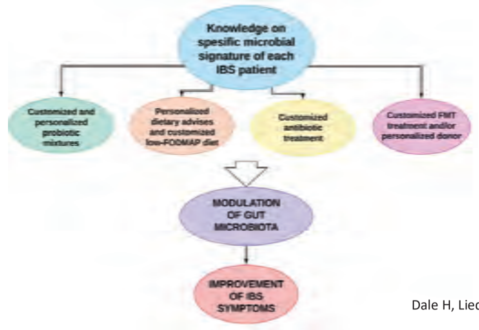
Current and proposed treatment strategies in microbe-based treatment for IBD

Mishima Y et al., J Gastroenterol 2020



protective bacterial strains
(**LBP**: Live biotherapeutic products)

Future therapeutic strategies in IBS



Dale H, Lied GA. Turk J Med Sci 2020

National Centre for Functional Gastrointestinal Disorders